

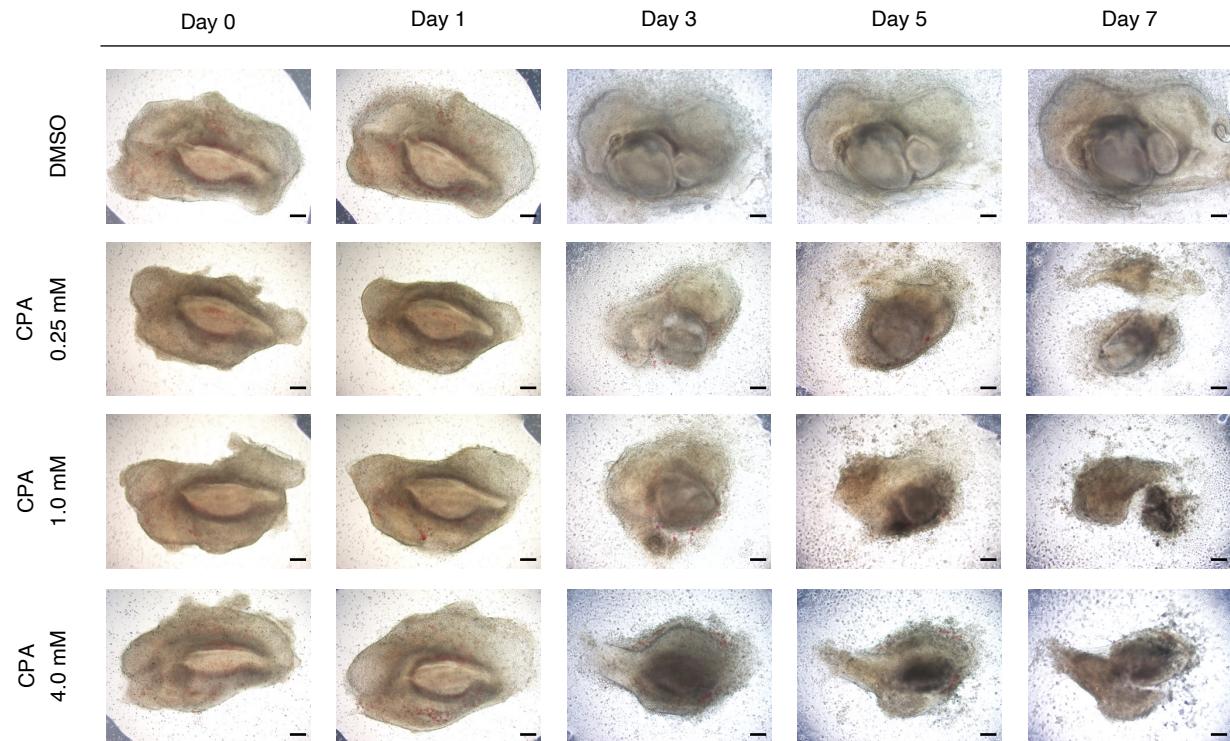
## Supplemental information

### **An ex vivo organ culture screening model revealed that low temperature conditions prevent side effects of anticancer drugs**

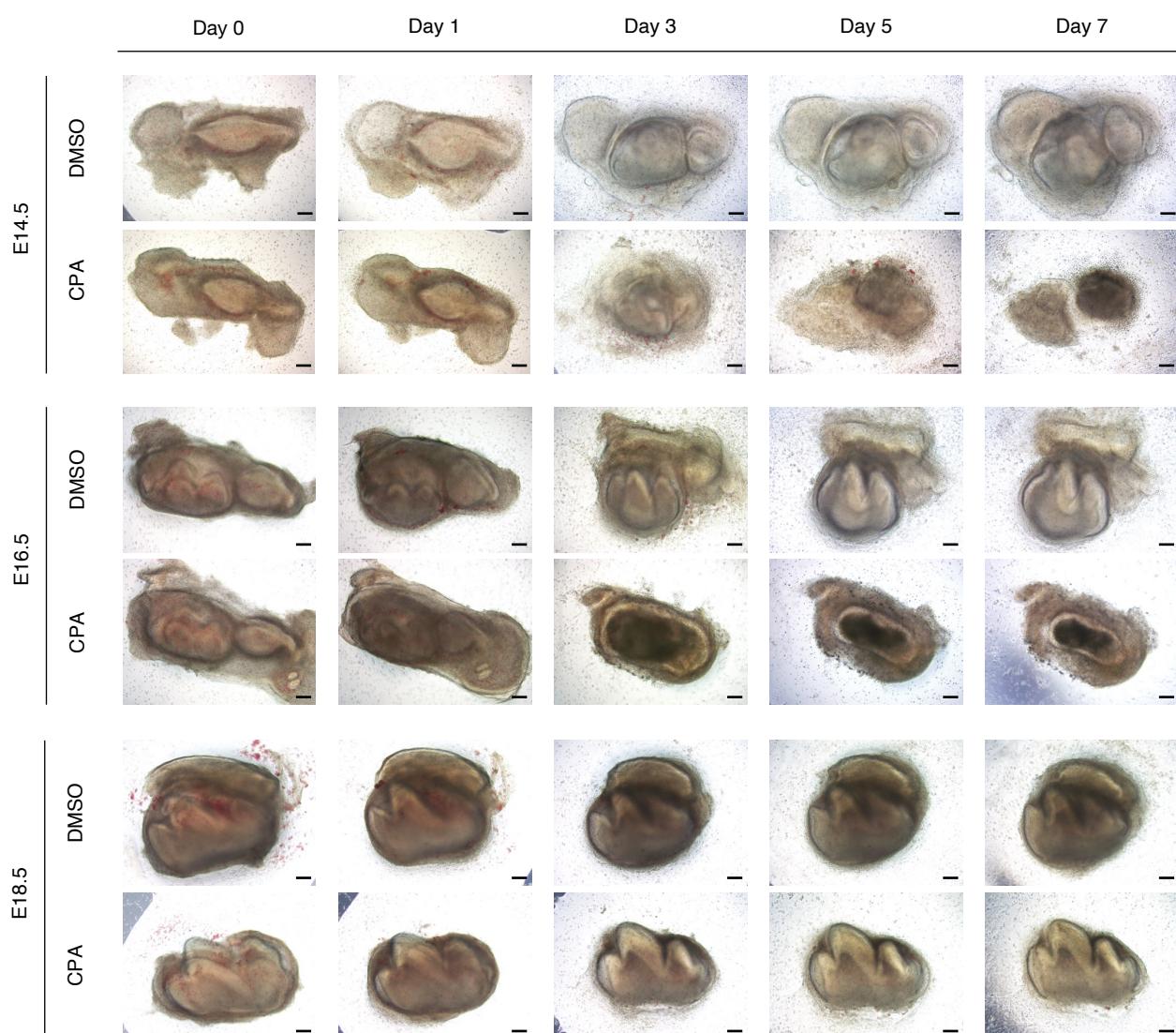
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Satoshi Fukumoto, Keigo Yoshizaki

# Supplementary Figure 1. Morphogenesis of tooth germs supplemented with CPA.

**a**



**b**

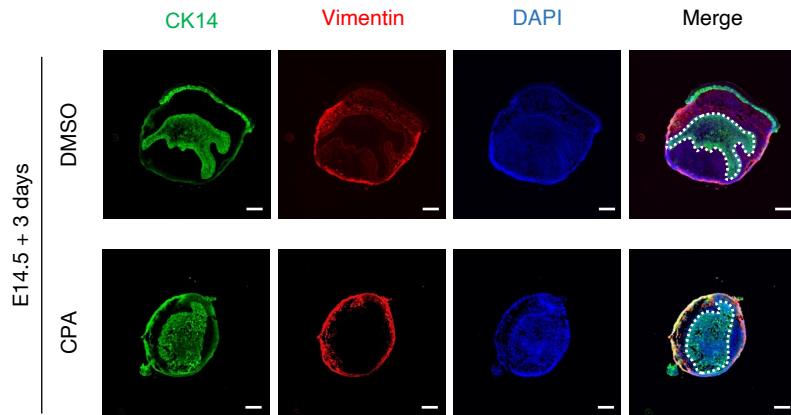


(a) Morphology of E14.5 tooth germs cultured for 0, 1, 3, 5 and 7-day in organ culture system, treated with DMSO or CPA (0.25 mM, 1.0 mM, 4.0 mM) ( $n = 10$ ).

(b) Morphology of different developmental stage of tooth germs cultured for 0, 1, 3, 5 and 7-day in organ culture system, treated with DMSO or 0.25 mM of CPA ( $n = 8$ ).

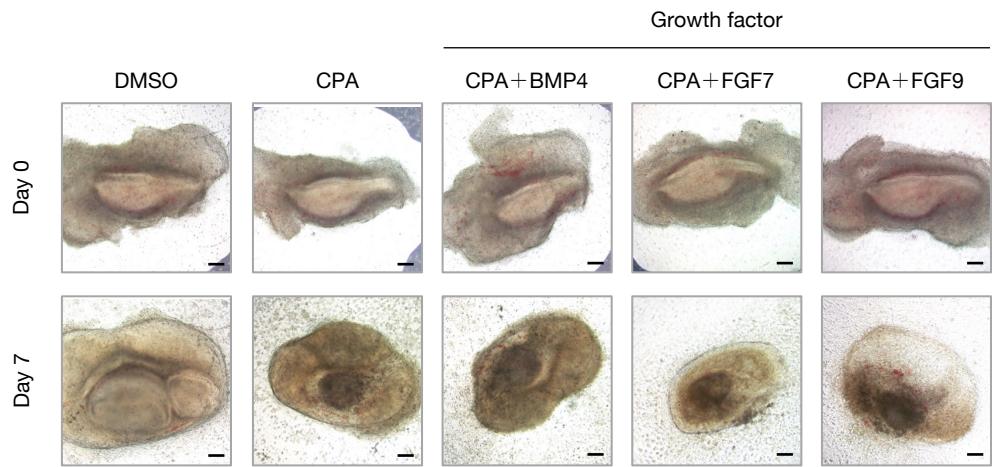
Scale bars, 200  $\mu$ m.

## Supplementary Figure 2. The expression of CK14 and vimentin in CPA-treated tooth germs.



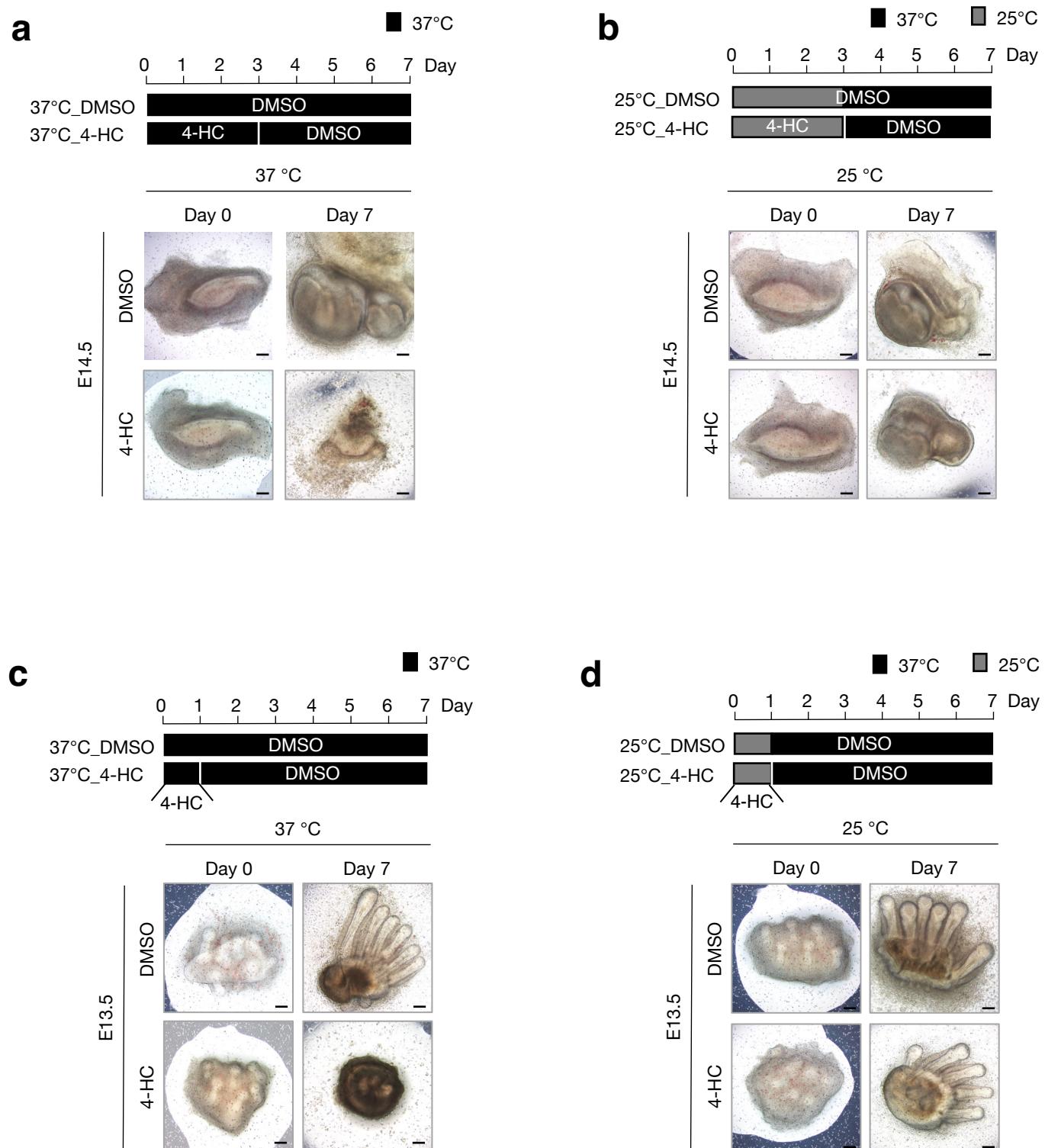
Immunofluorescence of CK14 (green) and Vimentin (red) in the section of E14.5 tooth germs cultured for three-day treated with DMSO or 0.25 mM of CPA. Nuclei were stained with DAPI (blue). Scale bars, 200  $\mu$ m.

**Supplementary Figure 3. The damaged tooth germs by CPA cannot be rescued by the addition of growth factors.**



Morphology of E14.5 tooth germs cultured for seven-day treated with DMSO, 0.25 mM of CPA, or 0.25 mM of CPA with growth factor (BMP4, FGF7, FGF9) ( $n = 8$ ). Scale bars, 200  $\mu$ m.

## Supplementary Figure 4. Low-temperature culture method decreases the 4-hydroxycyclophosphamide (4-HC)-mediated damage on the growth of culture organs.



(a) Morphology of tooth germs in a conventional method of organ culture. In the first three days, tooth germs were treated with DMSO or 1 $\mu$ M of 4-HC (Toronto Research Chemicals, Inc.), then the culturing medium was replaced to that of without 4-HC for additional four days. The temperature was maintained at 37°C throughout incubation of tooth germs.

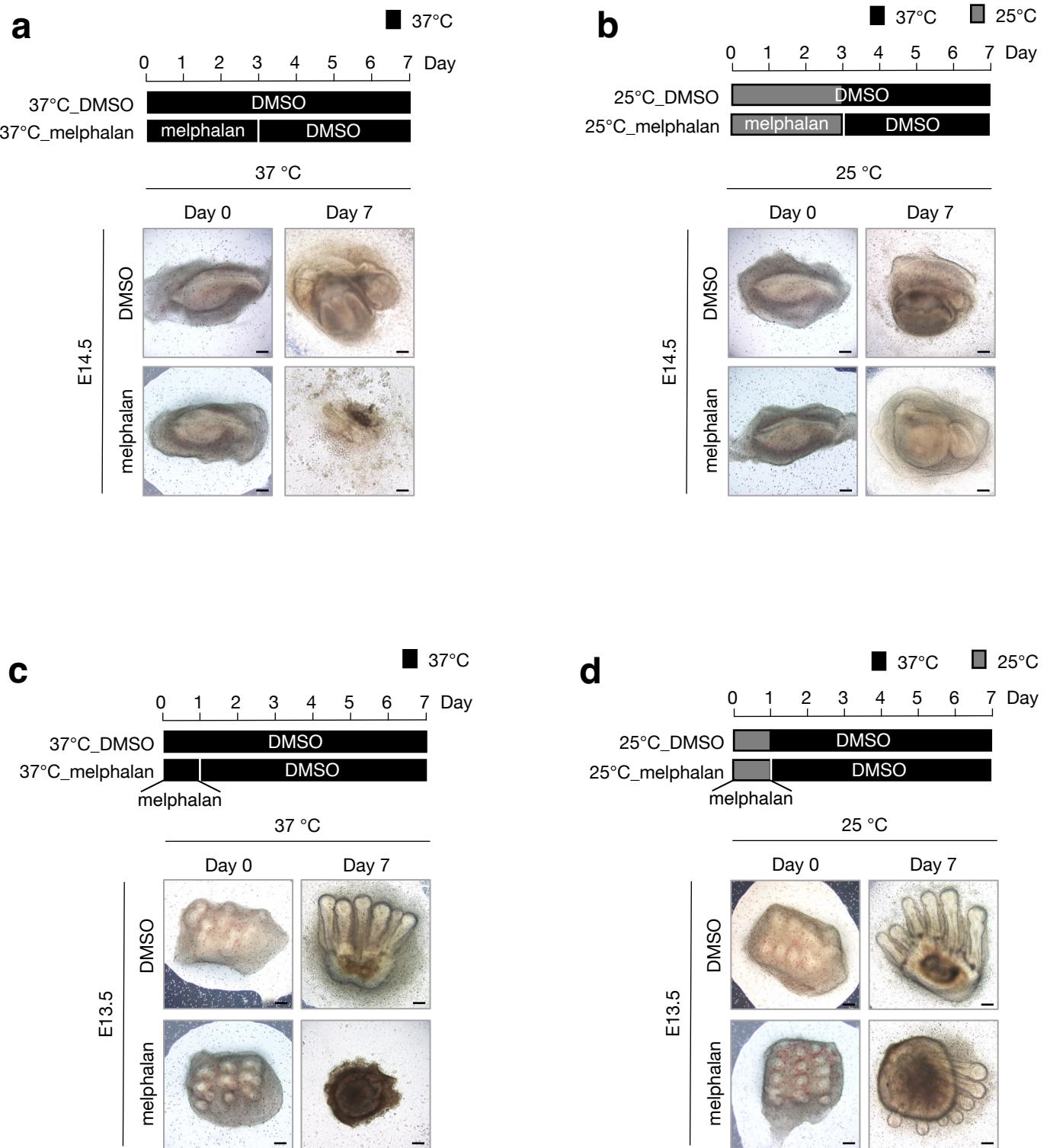
(b) Morphology of tooth germs in a low-temperature culture method of organ culture. Tooth germs were treated with DMSO or 1 $\mu$ M of 4-HC at 25°C in the first three days, then the culturing medium was replaced to that of without 4-HC for additional four days at 37°C.

(c) Organ cultured E13.5 hair tissues for 7 days with normal method.

(d) E13.5 hair tissues were organ cultured for 7 days with low-temperature method.

Scale bars, 200  $\mu$ m.

## Supplementary Figure 5. Low-temperature culture method decreases the melphalan-mediated damage on the growth of culture organs.



(a) Morphology of tooth germs in a conventional method of organ culture. In the first three days, tooth germs were treated with DMSO or 10  $\mu$ M of melphalan (Sigma, St. Louis, MS, USA), then the culturing medium was replaced to that of without melphalan for additional four days. The temperature was maintained at 37°C throughout incubation of tooth germs.

(b) Morphology of tooth germs in a low-temperature culture method of organ culture. Tooth germs were treated with DMSO or 10  $\mu$ M of melphalan at 25°C in the first three days, then the culturing medium was replaced to that of without melphalan for additional four days at 37°C.

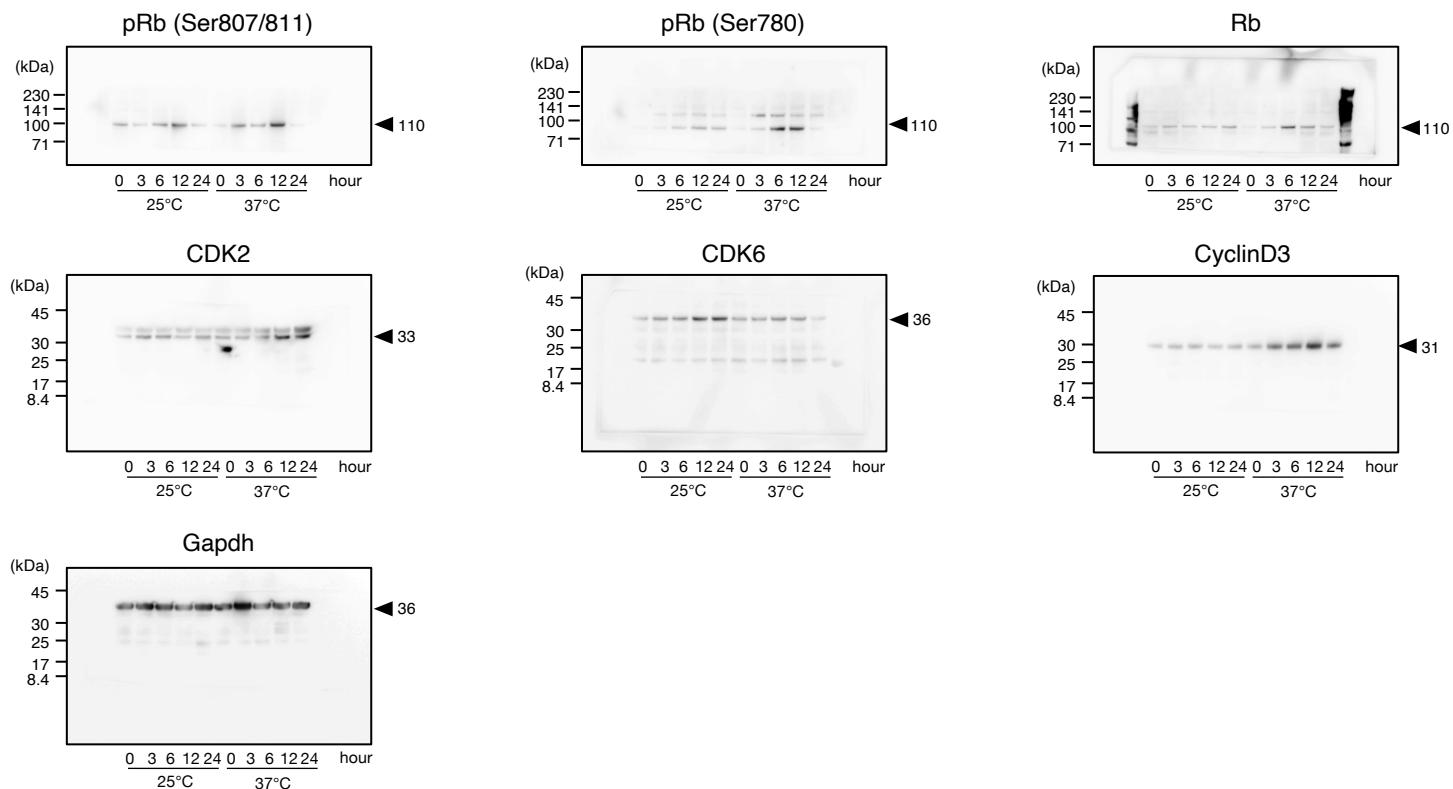
(c) Organ cultured E13.5 hair tissues for 7 days with normal method.

(d) E13.5 hair tissues were organ cultured for 7 days with low-temperature method.

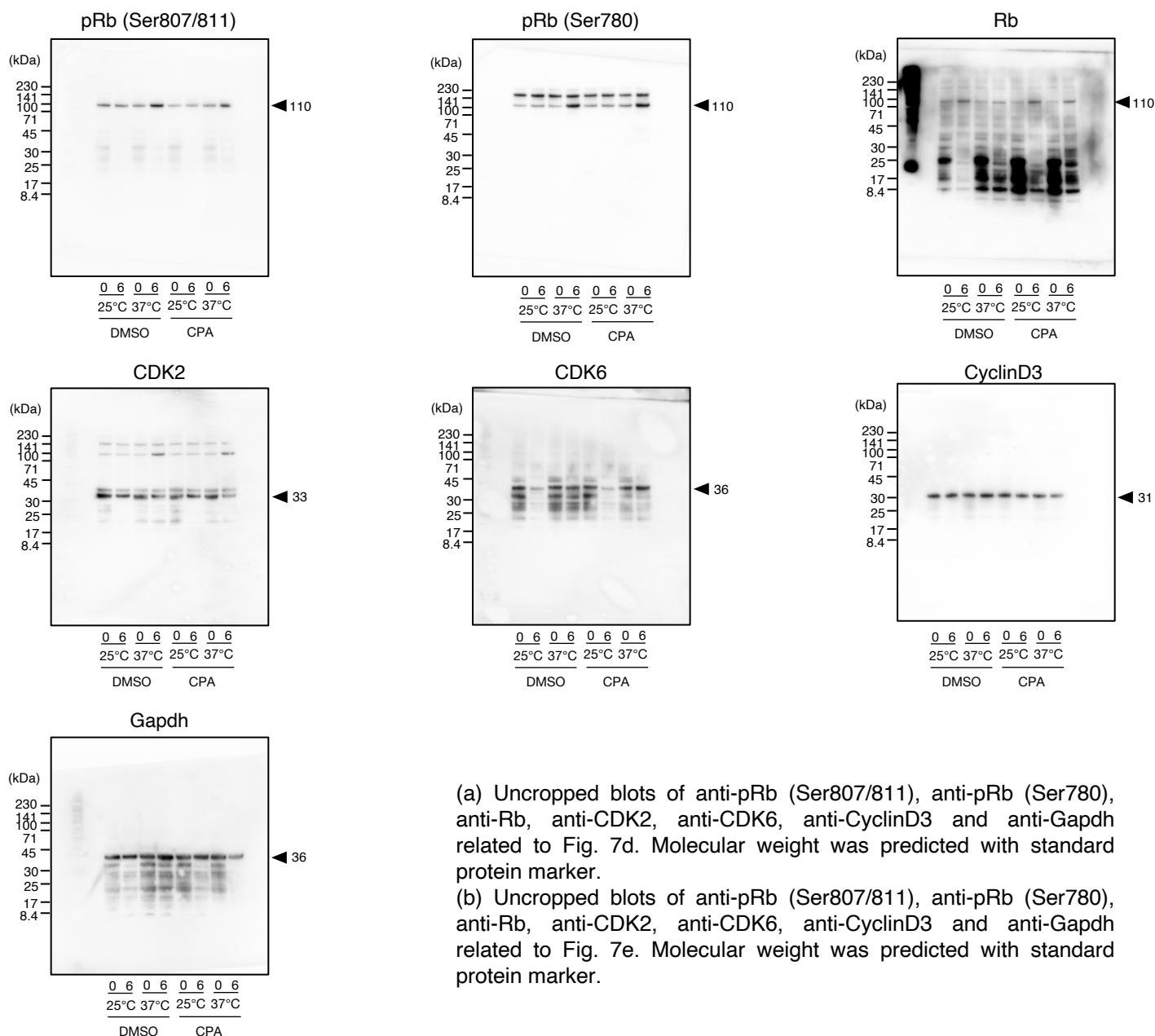
Scale bars, 200  $\mu$ m.

## Supplementary Figure 6. The uncropped blots presented in Fig. 7.

**a**



**b**



(a) Uncropped blots of anti-pRb (Ser807/811), anti-pRb (Ser780), anti-Rb, anti-CDK2, anti-CDK6, anti-CyclinD3 and anti-Gapdh related to Fig. 7d. Molecular weight was predicted with standard protein marker.

(b) Uncropped blots of anti-pRb (Ser807/811), anti-pRb (Ser780), anti-Rb, anti-CDK2, anti-CDK6, anti-CyclinD3 and anti-Gapdh related to Fig. 7e. Molecular weight was predicted with standard protein marker.

Supplementary Table 1. Full-list of gene ontology term related to Fig. 7b.

| GO biological process                                                 | list of genes |          |        |        |         |        |        |        |        |        |
|-----------------------------------------------------------------------|---------------|----------|--------|--------|---------|--------|--------|--------|--------|--------|
| regulation of double-strand break repair via homologous recombination | Tex15         | Rad51ap1 | Zfp365 | Rad51  |         |        |        |        |        |        |
| intrinsic apoptotic signaling pathway by p53 class mediator           | Bbc3          | Eda2r    | E2f2   | Aen    |         |        |        |        |        |        |
| mitotic DNA damage checkpoint                                         | Fancd2        | Eme1     | Ccng1  | Pidd1  |         |        |        |        |        |        |
| negative regulation of G1/S transition of mitotic cell cycle          | E2f7          | Plk5     | Pidd1  | Haspin |         |        |        |        |        |        |
| mitotic sister chromatid segregation                                  | Ncapg         | Kif18b   | Espl1  | Spag5  | Cdca5   | Psrc1  | Haspin |        |        |        |
| signal transduction in response to DNA damage                         | E2f7          | Sesn2    | Brca1  | Pidd1  |         |        |        |        |        |        |
| meiosis I                                                             | Fancd2        | Tex15    | Espl1  | Ccne2  | Eme1    | Mybl1  | Rad51  |        |        |        |
| double-strand break repair via homologous recombination               | Rad51ap1      | Brca1    | Aunip  | Cdc45  | Rad51   |        |        |        |        |        |
| meiotic chromosome segregation                                        | Fancd2        | Tex15    | Espl1  | Ccne2  | Eme1    |        |        |        |        |        |
| DNA replication                                                       | Rrm2          | Polk     | Ccne2  | Eme1   | Fam111a | Cdc45  | Rad51  |        |        |        |
| spindle organization                                                  | Espl1         | Kifc1    | Kifc5b | Spag5  | Aunip   | Kif11  |        |        |        |        |
| mitotic cell cycle phase transition                                   | Tcf19         | E2f7     | Ube2c  | Ccne2  | Ccng1   | Iqgap3 |        |        |        |        |
| cell division                                                         | Plk5          | Kif18b   | Ube2c  | Ccne2  | Kntc1   | Spag5  | Cdca5  | Ercc6l | Zwilch | Zfp365 |
|                                                                       | Ckap2         | Ccng1    | Psrc1  | Kif11  |         |        |        |        |        |        |
| positive regulation of cell cycle process                             | E2f7          | Ube2c    | Brca1  | Spag5  | Cdca5   | Pidd1  | Psrc1  | Fosl1  |        |        |

**Supplementary Table 2. Antibodies used in this study.**

| Antibody                                             | Purpose                    | Dilution | Provider                                    |
|------------------------------------------------------|----------------------------|----------|---------------------------------------------|
| Anti-Ki67                                            | Whole-mount immunostaining | 1:100    | Cell Signaling Technology, Danvers, MS, USA |
| Anti-Perlecan                                        | Whole-mount immunostaining | 1:100    | Invitrogen, Waltham, MS, USA                |
| Alexa 488 or Alexa 594 fluorescent dye               | Whole-mount immunostaining | 1:250    | Life Technologies, Waltham, MS, USA         |
| Anti-Ki67                                            | Immunostaining             | 1:500    | Cell Signaling Technology, Danvers, MS, USA |
| Anti-Perlecan                                        | Immunostaining             | 1:500    | Invitrogen, Waltham, MS, USA                |
| Anti-p21                                             | Immunostaining             | 1:500    | abcam, Cambridge, UK                        |
| Anti- $\gamma$ -H2AX                                 | Immunostaining             | 1:500    | Cell Signaling Technology, Danvers, MS, USA |
| Anti-Keratin 14                                      | Immunostaining             | 1:500    | Covance, Princeton, NJ, USA                 |
| Anti-Vimentin                                        | Immunostaining             | 1:500    | Santa Cruz, Dallas, TX, USA                 |
| Alexa 488 or Alexa 594 fluorescent dye               | Immunostaining             | 1:500    | Life Technologies, Waltham, MS, USA         |
| Anti-pRb(Ser807/811)                                 | Western blotting           | 1:500    | Cell Signaling Technology, Danvers, MS, USA |
| Anti-pRb(Ser780)                                     | Western blotting           | 1:500    | Cell Signaling Technology, Danvers, MS, USA |
| Anti-Rb                                              | Western blotting           | 1:500    | Cell Signaling Technology, Danvers, MS, USA |
| Anti-CDK2                                            | Western blotting           | 1:500    | Cell Signaling Technology, Danvers, MS, USA |
| Anti-CDK6                                            | Western blotting           | 1:500    | Cell Signaling Technology, Danvers, MS, USA |
| Anti-CyclinD3                                        | Western blotting           | 1:500    | Cell Signaling Technology, Danvers, MS, USA |
| Anti-GAPDH                                           | Western blotting           | 1:500    | Cell Signaling Technology, Danvers, MS, USA |
| Horseradish peroxidase-conjugated secondary antibody | Western blotting           | 1:1000   | Cell Signaling Technology, Danvers, MS, USA |

Supplementary Table 3. Primer sequences used in this study.

| Gene                                                           | Forward                      | Reverse                     |
|----------------------------------------------------------------|------------------------------|-----------------------------|
| <i>Amelogenin</i>                                              | 5'-ctgtctcaacagcatccc -3'    | 5'-caggaactggcatcatg-3'     |
| <i>Ameloblastin (Ambn)</i>                                     | 5'-acaacgcatggcgttccaa-3'    | 5'-accttcactgcggaggata-3'   |
| <i>AmeloD</i>                                                  | 5'-actacgacgcctacactggg-3'   | 5'-atgaaggcaggctcgAACGG-3'  |
| <i>Nkx2-3</i>                                                  | 5'-tggccctgatgttttacca-3'    | 5'-ggaaaaactgcgtcccttcag-3' |
| <i>p21</i>                                                     | 5'-tcccgactcttgacattgct-3'   | 5'-tccaaaatagaggggcagct-3'  |
| <b>glyceraldehyde 3-phosphate dehydrogenase (<i>Gapdh</i>)</b> | 5'-ggagcgagaccccactaacatc-3' | 5'-ctcggttcacacccatcac-3'   |

Supplementary Table 4. Full-list of genes upregulated in 37°C\_CPA.

| Gene                 | 25_DMSO    |            | 25_CPA |            | 37_DMSO    |      | 37_CPA     |    |
|----------------------|------------|------------|--------|------------|------------|------|------------|----|
|                      | raw signal | raw signal | FC     | raw signal | raw signal | FC   | raw signal | FC |
| <i>Tcf19</i>         | 181        | 183        | 1.0    | 227        | 502        | 2.2  |            |    |
| <i>H2-Eb1</i>        | 7          | 1          | 0.1    | 13         | 82         | 6.3  |            |    |
| <i>Eda2r</i>         | 60         | 55         | 0.9    | 64         | 442        | 6.9  |            |    |
| <i>Spag5</i>         | 49         | 45         | 0.9    | 69         | 158        | 2.3  |            |    |
| <i>Rhbd2</i>         | 44         | 47         | 1.1    | 62         | 140        | 2.3  |            |    |
| <i>Adrb2</i>         | 31         | 42         | 1.4    | 53         | 122        | 2.3  |            |    |
| <i>Mefl</i>          | 181        | 172        | 1.0    | 241        | 480        | 2.0  |            |    |
| <i>Eme1</i>          | 66         | 74         | 1.1    | 108        | 235        | 2.2  |            |    |
| <i>Gm13067</i>       | 52         | 50         | 1.0    | 38         | 319        | 8.4  |            |    |
| <i>Cdc42bp</i>       | 128        | 167        | 1.3    | 205        | 545        | 2.7  |            |    |
| <i>Inka2</i>         | 44         | 40         | 0.9    | 79         | 428        | 5.4  |            |    |
| <i>Hnob</i>          | 69         | 57         | 0.8    | 90         | 192        | 2.1  |            |    |
| <i>Gm25791</i>       | 10         | 2          | 0.2    | 37         | 79         | 2.1  |            |    |
| <i>9230114K14Rik</i> | 52         | 66         | 1.3    | 48         | 189        | 3.9  |            |    |
| <i>Fam124a</i>       | 81         | 90         | 1.1    | 35         | 171        | 4.9  |            |    |
| <i>Cox6b2</i>        | 133        | 150        | 1.1    | 226        | 1059       | 4.7  |            |    |
| <i>Kif1b</i>         | 241        | 242        | 1.0    | 271        | 684        | 2.5  |            |    |
| <i>Gm23935</i>       | 2985       | 3170       | 1.1    | 2746       | 8958       | 3.3  |            |    |
| <i>Sfn9</i>          | 106        | 110        | 1.0    | 110        | 284        | 2.6  |            |    |
| <i>Ncapg</i>         | 388        | 420        | 1.1    | 545        | 1177       | 2.2  |            |    |
| <i>Pidd1</i>         | 85         | 105        | 1.2    | 72         | 318        | 4.4  |            |    |
| <i>Krtc1</i>         | 106        | 119        | 1.1    | 141        | 396        | 2.8  |            |    |
| <i>Bbc3</i>          | 158        | 125        | 0.8    | 185        | 410        | 2.2  |            |    |
| <i>Ano3</i>          | 14         | 10         | 0.7    | 4          | 214        | 53.5 |            |    |
| <i>Sic19a2</i>       | 334        | 360        | 1.1    | 283        | 1012       | 3.6  |            |    |
| <i>Trp53inp1</i>     | 227        | 243        | 1.1    | 209        | 1063       | 5.1  |            |    |
| <i>Kif11</i>         | 380        | 352        | 0.9    | 569        | 1050       | 1.8  |            |    |
| <i>Ephx1</i>         | 102        | 88         | 0.9    | 198        | 1029       | 5.2  |            |    |
| <i>Gm2579</i>        | 10         | 2          | 0.2    | 37         | 79         | 2.1  |            |    |
| <i>Dgucy</i>         | 78         | 70         | 0.9    | 85         | 353        | 4.2  |            |    |
| <i>1500009L16Rik</i> | 182        | 150        | 0.8    | 286        | 683        | 2.4  |            |    |
| <i>Mns1</i>          | 35         | 30         | 0.9    | 35         | 92         | 2.6  |            |    |
| <i>Cone2</i>         | 477        | 428        | 0.9    | 550        | 1635       | 3.0  |            |    |
| <i>Neil3</i>         | 185        | 212        | 1.1    | 233        | 580        | 2.5  |            |    |
| <i>Erc6l</i>         | 110        | 133        | 1.2    | 154        | 342        | 2.2  |            |    |
| <i>Gm27032</i>       | 0          | 1          | 0.0    | 35         | 163        | 4.7  |            |    |
| <i>Zfp365</i>        | 105        | 139        | 1.3    | 65         | 284        | 4.4  |            |    |
| <i>Kif5b</i>         | 100        | 105        | 1.1    | 93         | 295        | 3.2  |            |    |
| <i>2310008N11Rik</i> | 22         | 15         | 0.7    | 9          | 110        | 12.2 |            |    |
| <i>Mir124a-3</i>     | 0          | 1          | 0.0    | 35         | 163        | 4.7  |            |    |
| <i>Btg2</i>          | 431        | 528        | 1.2    | 795        | 1967       | 2.5  |            |    |
| <i>1700007K13Rik</i> | 61         | 76         | 1.2    | 84         | 532        | 6.3  |            |    |
| <i>Igap3</i>         | 74         | 72         | 1.0    | 115        | 224        | 1.9  |            |    |
| <i>Fancd2</i>        | 83         | 90         | 1.1    | 106        | 295        | 2.8  |            |    |
| <i>Slc2a9</i>        | 88         | 98         | 1.1    | 71         | 214        | 3.0  |            |    |
| <i>Tex15</i>         | 21         | 19         | 0.9    | 43         | 159        | 3.7  |            |    |
| <i>Exo1</i>          | 152        | 144        | 0.9    | 126        | 388        | 3.1  |            |    |
| <i>Rrm2</i>          | 455        | 454        | 1.0    | 430        | 1332       | 3.1  |            |    |
| <i>Gtse1</i>         | 198        | 229        | 1.2    | 385        | 1302       | 3.4  |            |    |
| <i>Abcb1b</i>        | 17         | 20         | 1.2    | 29         | 370        | 12.8 |            |    |
| <i>Kifc1</i>         | 204        | 247        | 1.2    | 303        | 757        | 2.5  |            |    |
| <i>Brca1</i>         | 173        | 222        | 1.3    | 203        | 571        | 2.8  |            |    |
| <i>Gm22620</i>       | 10         | 2          | 0.2    | 37         | 79         | 2.1  |            |    |
| <i>Gm17511</i>       | 3276       | 4628       | 1.4    | 120        | 5326       | 44.4 |            |    |
| <i>Bard1</i>         | 205        | 193        | 0.9    | 257        | 590        | 2.3  |            |    |
| <i>Gm3776</i>        | 7          | 4          | 0.6    | 14         | 103        | 7.4  |            |    |
| <i>Tnfrsf10b</i>     | 301        | 302        | 1.0    | 277        | 758        | 2.7  |            |    |
| <i>Polk</i>          | 224        | 190        | 0.8    | 338        | 1034       | 3.1  |            |    |
| <i>Rnf169</i>        | 27         | 38         | 1.4    | 29         | 100        | 3.4  |            |    |
| <i>Ezr2</i>          | 35         | 42         | 1.2    | 44         | 140        | 3.2  |            |    |
| <i>Gipc2</i>         | 20         | 8          | 0.4    | 8          | 90         | 11.3 |            |    |
| <i>Mtf2</i>          | 100        | 125        | 1.3    | 95         | 262        | 2.8  |            |    |
| <i>Rad51</i>         | 539        | 588        | 1.1    | 502        | 1625       | 3.0  |            |    |
| <i>Sesn2</i>         | 286        | 283        | 1.0    | 312        | 968        | 3.1  |            |    |
| <i>Dafas</i>         | 219        | 213        | 1.0    | 134        | 912        | 6.8  |            |    |
| <i>Fanci</i>         | 53         | 65         | 1.2    | 85         | 224        | 2.6  |            |    |
| <i>Pfk5</i>          | 4          | 8          | 2.0    | 18         | 311        | 17.3 |            |    |
| <i>Ube2c</i>         | 1315       | 1344       | 1.0    | 2086       | 4095       | 2.0  |            |    |
| <i>Fosl1</i>         | 57         | 65         | 1.1    | 85         | 183        | 2.2  |            |    |
| <i>Tubb4b</i>        | 1678       | 1727       | 1.0    | 2229       | 5256       | 2.4  |            |    |
| <i>Esco2</i>         | 254        | 233        | 0.9    | 361        | 824        | 2.3  |            |    |
| <i>Gm10073</i>       | 112        | 156        | 1.4    | 4          | 223        | 55.8 |            |    |
| <i>Psrc1</i>         | 228        | 210        | 0.9    | 348        | 1332       | 3.8  |            |    |
| <i>Ckap2</i>         | 345        | 375        | 1.1    | 360        | 1030       | 2.9  |            |    |
| <i>Gm12981</i>       | 286        | 283        | 1.0    | 312        | 968        | 3.1  |            |    |
| <i>Centri</i>        | 76         | 95         | 1.3    | 71         | 197        | 2.8  |            |    |
| <i>Amtl2</i>         | 5          | 14         | 2.8    | 46         | 213        | 4.6  |            |    |
| <i>Cdc45</i>         | 156        | 156        | 1.0    | 214        | 428        | 2.0  |            |    |
| <i>Cmpk2</i>         | 20         | 26         | 1.3    | 42         | 85         | 2.0  |            |    |
| <i>Gm49349</i>       | 10         | 2          | 0.2    | 37         | 79         | 2.1  |            |    |
| <i>Celf5</i>         | 20         | 30         | 1.5    | 53         | 339        | 6.4  |            |    |
| <i>Acaa1b</i>        | 120        | 132        | 1.1    | 79         | 1030       | 13.0 |            |    |
| <i>Cng1</i>          | 1213       | 1222       | 1.0    | 1052       | 4878       | 4.6  |            |    |
| <i>Steap1</i>        | 20         | 11         | 0.6    | 36         | 76         | 2.1  |            |    |
| <i>Haspin</i>        | 55         | 50         | 0.9    | 53         | 154        | 2.9  |            |    |
| <i>Fam111a</i>       | 151        | 185        | 1.2    | 252        | 503        | 2.0  |            |    |
| <i>Ankle1</i>        | 63         | 69         | 1.1    | 105        | 231        | 2.2  |            |    |
| <i>Trim7</i>         | 8          | 1          | 0.1    | 26         | 176        | 6.8  |            |    |
| <i>Dcxr</i>          | 225        | 229        | 1.0    | 105        | 780        | 7.4  |            |    |
| <i>Aunip</i>         | 70         | 44         | 0.6    | 95         | 251        | 2.6  |            |    |
| <i>Cdc45</i>         | 362        | 302        | 0.8    | 522        | 994        | 1.9  |            |    |
| <i>Scn1b</i>         | 13         | 11         | 0.8    | 27         | 75         | 2.8  |            |    |
| <i>Gm10221</i>       | 50         | 2          | 0.0    | 21         | 271        | 12.9 |            |    |
| <i>Kif15</i>         | 213        | 175        | 0.8    | 296        | 824        | 2.8  |            |    |
| <i>Capsl</i>         | 15         | 9          | 0.6    | 30         | 91         | 3.0  |            |    |
| <i>Gm5424</i>        | 231        | 207        | 0.9    | 323        | 791        | 2.4  |            |    |
| <i>Nkr2-9</i>        | 0          | 2          | 0.0    | 0          | 58         | 0.0  |            |    |
| <i>Tnfrsf18</i>      | 25         | 38         | 1.5    | 51         | 243        | 4.8  |            |    |
| <i>A930001C03Rik</i> | 32         | 30         | 0.9    | 24         | 186        | 7.8  |            |    |
| <i>Ass1</i>          | 32         | 32         | 1.0    | 39         | 121        | 3.1  |            |    |
| <i>Rad51ap1</i>      | 113        | 148        | 1.3    | 191        | 398        | 2.1  |            |    |
| <i>Shcbp1</i>        | 127        | 139        | 1.1    | 201        | 429        | 2.1  |            |    |
| <i>Mgmr1</i>         | 268        | 206        | 0.8    | 156        | 506        | 3.2  |            |    |
| <i>Aen</i>           | 727        | 787        | 1.1    | 665        | 2011       | 3.0  |            |    |
| <i>Rpm</i>           | 49         | 43         | 0.9    | 29         | 105        | 3.6  |            |    |
| <i>Hmgcl1</i>        | 30         | 52         | 1.7    | 74         | 192        | 2.6  |            |    |
| <i>Zwilch</i>        | 152        | 122        | 0.8    | 100        | 316        | 3.2  |            |    |
| <i>Gm23455</i>       | 10         | 2          | 0.2    | 37         | 79         | 2.1  |            |    |
| <i>Exoc4</i>         | 322        | 331        | 1.0    | 372        | 1131       | 3.0  |            |    |
| <i>Zmat3</i>         | 300        | 256        | 0.9    | 279        | 1030       | 3.7  |            |    |
| <i>Fbxo48</i>        | 42         | 39         | 0.9    | 41         | 136        | 3.3  |            |    |
| <i>Svop</i>          | 2          | 3          | 1.5    | 0          | 63         | 0.0  |            |    |
| <i>Lrrf</i>          | 133        | 148        | 1.1    | 107        | 341        | 3.2  |            |    |
| <i>Mybl1</i>         | 90         | 100        | 1.1    | 82         | 443        | 5.4  |            |    |
| <i>Msa410</i>        | 14         | 10         | 0.7    | 3          | 122        | 40.7 |            |    |
| <i>Esp11</i>         | 73         | 63         | 0.9    | 116        | 273        | 2.4  |            |    |
| <i>Ezrf</i>          | 169        | 163        | 1.0    | 259        | 552        | 2.1  |            |    |
| <i>Scn3b</i>         | 37         | 36         | 1.0    | 15         | 89         | 5.9  |            |    |
| <i>Igsf9b</i>        | 45         | 53         | 1.2    | 69         | 297        | 4.3  |            |    |
| <i>Gm24357</i>       | 10         | 2          | 0.2    | 37         | 79         | 2.1  |            |    |
| <i>Eldr</i>          | 112        | 117        | 1.0    | 157        | 348        | 2.2  |            |    |